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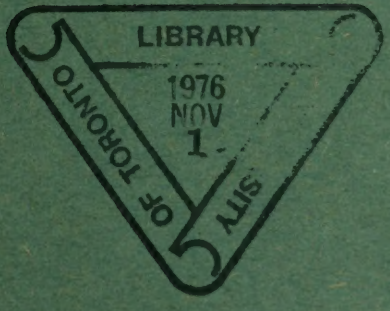


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TRANSACTIONS

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Proceedings of a Meeting of the Society held on Friday, February 17th, 1919, at 5.30 p.m., at 11, Chandos Street, Cavendish Square, W. 1., Surgeon-General Sir DAVID BRUCE, K.C.B., F.R.S. (*President*), in the Chair.

# THE MINUTE BODIES (RICKETTSIA) FOUND IN ASSOCIATION WITH TRENCH FEVER, TYPHUS FEVER AND ROCKY MOUNTAIN SPOTTED FEVER.

BY J. A. ARKWRIGHT, M.D., A. BACOT, AND F. M. DUNCAN.

*From the Lister Institute.*

Our work on Rickettsia has been done in connection with the War Office Committee on Trench Fever, under the chairmanship of Major-General Sir DAVID BRUCE, F.R.S., and in co-operation with Major BYAM, R.A.M.C., and the staff of the New End Hospital, Hampstead, to whom we are greatly indebted, and especially to Lieut. LLOYD, R.A.M.C., Entomologist to the Hospital.

The minute bodies to which the name "Rickettsia" was given by DA ROCHA-LIMA in 1916 are described in association with three diseases of man. They are most readily found in the invertebrate agent which transmits the disease, namely, *Pediculus corporis* or *capitis* in the cases of typhus and trench fevers, and the tick, *Dermacentor venustus*, in the case of Rocky Mountain fever. Another species is described by NÖLLER (1917) in the sheep-louse or "ked," *Melophagus ovinus*. TÖPFER (1917) claimed that Rickettsia of a distinguishable type was found in lice from cases of war nephritis, but this observation has not been confirmed.

RICKETTS (1909) was the first to describe these bodies in the blood of patients suffering from Rocky Mountain fever, and he also found them in ticks which had been fed on these patients.

RICKETTS and WILDER (1910) described very similar forms in the blood of cases of typhus and in lice taken from typhus patients. It is remarkable that they were first seen and noted in human blood films, though only small numbers have ever been found in such films, and there is so little distinctive in their appearance that they were at first not seriously recognised as competitors for the place of causal micro-organism. The attention that has been given to Rickettsia during the last three years is more dependent on its

occurrence in large numbers in the louse or tick than on the forms seen in blood films. TÖPFER (1916) was the first to record the association of Rickettsia in lice with trench fever.

### THE MORPHOLOGY AND APPEARANCE OF RICKETTSIA IN STAINED FILMS.

These minute organisms resemble very small bacteria, and are usually either round, oval, diplococcal or lancet-shaped. Sometimes, when double, the two parts are separated by an unstained central segment, when they resemble a dumbbell, or they may appear like short rods with the stain concentrated at the ends, or at three or even four points in their length; one half of a diplococcal form may be much bigger than the other. They may be arranged in pairs as a short chain of three to five links, placed in a curve or L shape. The range of forms is very like that of the *B. influenzæ*. In size they vary somewhat, especially in length, but they usually appear when stained to be about 0·2 to 0·3 microns in their shorter diameter by 0.3 to 0.5 in length. They are distinctly smaller than the *B. influenzæ* or *M. melitensis* from young cultures when these are stained side by side in the same film, and they are not so uniform in size as the latter organism.

Rickettsia is easily decolourised by alcohol in Gram's method of staining, and takes most stains feebly. It is best stained by Giemsa (one drop to the cubic centimetre) used for 16 to 20 hours.

Preliminary washing of the film with ether or fixing in ether and alcohol does not materially improve the staining by Gram's or other methods.

By Giemsa's stain the trench-fever Rickettsia is coloured, as a rule in our experience, purple or reddish purple like most bacteria. We have found it an advantage, when examining films made from the excreta of lice or from the guts of lice which contain much fresh or altered blood, to fix the film in absolute alcohol, containing 1 per cent. strong hydrochloric acid. By this means much of the débris of the red corpuscles is removed.

The forms which we have found in lice fed on human beings, or monkeys infected with typhus fever, sometimes appear slightly larger and more bacillary in shape; they also, as a rule, stain with Giemsa a redder or pinker colour than trench fever Rickettsia.

In films made from ticks infected with Rocky Mountain fever which we have been able to examine through the kindness of Dr. WOLBACH, of Harvard University, the bodies appear slightly larger, and more lancet-like or bacillary in shape.

We have found that the best routine method of examining lice which have been fed on trench fever patients with a view to determining whether they are infected with Rickettsia is to make a film of the excreta of the lice. This appears to have been very little done by other observers, who have worked almost entirely by cutting sections of lice or making films of their intestinal contents We have not so readily found Rickettsia in the excreta of lice which have fed on typhus patients.

Examining the excreta enables observations to be made on a box of lice for a number of consecutive days without destroying the lice. The lice with which we have worked have been kept in boxes with a gauze cover, through which they feed and through which the excreta can be daily shaken out.

In films shewing Rickettsia, whether of excreta or single lice, the micro-organisms are often seen in enormous numbers, and may be in masses which appear to consist of nothing else. Often they are seen embedded in flakes of semitranslucent material, which is, perhaps, mucus or part of a disintegrated cell. Under these latter conditions the Rickettsia stains well, and shews a very definite outline lying in the feebly stained matrix. In sections of lice the same appearance is seen in parts of the gut where the contents are reduced in bulk, and the blood débris has almost disappeared.

The most distinctive characters of Rickettsia in lice and their excreta are the size, number and staining properties, besides the shape and arrangement. The last two characters appear to us to be insufficient alone for the identification of these bodies when they are few in number and scattered singly in a film. Granules of similar size and shape are often to be seen in films of the excreta of normal lice or of healthy blood which has been for some time in the incubator; they can usually be recognised by their shadowy appearance, feeble staining and indefinite margins. It is not intended to convey the impression that the Rickettsia is always easy to recognise in films of louse excreta or gut contents. Sometimes a stained precipitate or deposit of stain may cause much uncertainty.

However, in a very large proportion of infected louse excreta, or in infected lice, the numbers and typical appearance leave no room for doubt. We have always recorded the result as negative in doubtful cases.

### Regularity of the Association of Rickettsia in Lice with Trench Fever in Man.

Our work has been chiefly done with trench fever, but we have made a few observations on typhus, which will be mentioned later.

We have been much struck with the great regularity with which Rickettsia appears in the excreta of lice when these are examined daily after feeding on a trench fever patient. The first appearance is, as a rule, eight to ten days after the first infecting feed. The occurrence is extraordinarily regular if the lice are fed twice daily on the same infected man, beginning on the first or second day of the disease. A like result usually follows feeding on an infected man for one day only, and subsequently on a healthy man. Lice do not, however, necessarily become infected with Rickettsia every such day on which they feed, and not all of those fed two or three times shew Rickettsia.

These results are well shown by reference to an experiment carried out on two volunteers—Purton, who was experimentally infected with trench fever, and a healthy man, Elliot. Purton was inoculated with the excreta of infected lice.

This experiment was carried out with great care and no little trouble at the New End Hospital, Hampstead, by Lieut. Lloyd, to whom we are very much indebted, and the lice and excreta were examined at the Lister Institute by the present writers.

A fresh box of about fifty healthy lice was fed on Purton every day, and subsequently fed daily on Elliott. All the lice used were from the same healthy stock, and each box contained lice of similar age. This course of feeding was begun on the day on which Purton was inoculated and was continued for a fortnight, *i.e.*, through six days of incubation before Purton became feverish until the eighth day of Purton's disease. It was preceded by feeding fifty lice in another box on Purton for the five consecutive days immediately before the inoculation, as a control to shew that he was not in a condition to infect lice with Rickettsia before the inoculation. In addition, two boxes were fed on Purton on the first day of his illness, and twice daily on him subsequently. The lice were contained in gauze-covered boxes, and during feeding were bandaged on to the forearm for thirty minutes.

All the lice were kept in an incubator at 30° C. between their feeds

In this experiment twenty boxes of lice were used and examined daily for three weeks.

TABLE I.

| Date. | Fed on | No. of Box. | Duration of feeding. | Appearance of R. in excreta. | Duration of R. in excreta. | Proportion of infected lice. |
|---|---|---|---|---|---|---|
| Control | | | | | | |
| 22 x. to 20 xi. | E | 260 | 33 days | 0 | 0 | nil |
| 21 to 25 x. | P | 261 | 4 ,, | 0 | 0 | nil |
| 26 x. | P inoculated | 262 | 1 ,, | 0 | 0 | nil |
| 27 x. | P | 263 | 1 ,, | 0 | 0 | nil |
| 28 x. | P | 264 | 1 ,, | 0 | 0 | nil |
| 29 x. | P | 265 | 1 ,, | 0 | 0 | nil |
| 30 x. | P | 266 | 1 ,, | 0 | 0 | nil |
| 31 x. | P | 267 | 1 ,, | 0 | 0 | nil |
| 1 xi. | P | 268 | 1 ,, | twelfth day | 2 days | 0 in 6 = 0 % |
| 2 xi. | Onset of fever | | | | | |
| 2, 3 xi. | P | 269 | 2 ,, | 0 | 0 | nil |
| 2 xi. | P | 270 | 1 ,, | 0 | 0 | nil |
| 3 xi. | P | 271 | 1 ,, | ninth day | 6 days | 1 in 37 = 2·7 % |
| 4 xi. | P | 272 | 1 ,, | 0 | 0 | nil |
| 5 xi. | P | 273 | 1 ,, | eighth day | 14 days | 6 in 27 = 27 % |
| 6 xi. | P | 274 | 1 ,, | ninth day | 12 ,, | 3 in 24 = 12·5 % |
| 7 xi. | P | 275 | 1 ,, | eighth day | 20 ,, | 12 in 41 = 28·6 % |
| 8 xi. | P | 276 | 1 ,, | seventh day | 15 ,, | 1 in 44 = 2·3 % |
| 9 xi. | P | 277 | 1 ,, | eleventh day | 15 ,, | 9 in 28 = 32 % |
| 2 to 18 xi. | P | 258 | 16 ,, | ninth day | more than 8 | —— |
| 2 to 26 xi. | P | 280 | 23 ,, | ninth day | ,, ,, 11 | 13 in 16 = 81 % |

Table I. and Chart I. shew the results obtained by examining films made from the excreta in the different boxes daily.

It is seen that the excreta of the lice fed during the four days before inoculation and from the day of inoculation up to the fifth day of incubation never shewed Rickettsia. On the other hand, seven of the ten boxes of lice fed on Purton on one day only each from the sixth day of incubation to the eighth day from the febrile onset did shew Rickettsia after the lapse of seven to twelve days from their feed on Purton. Also the excreta from both boxes, Nos. 258 and 280, fed twice daily on the infected man shewed Rickettsia on the ninth day from the first feed on him.

To sum up, most of the boxes of lice fed on this trench fever patient early in the disease became infected with Rickettsia after an interval which may be called the incubation period in the louse, but the lice in similar boxes fed on the same man before his illness or on a healthy man did not excrete Rickettsia. When the results of feeding lice on different days are examined in detail it is seen that those feeding on some days during the illness became infected, but those feeding on other days did not. Moreover it was not necessary that the patient should be febrile in order that the lice should become infected; indeed in this series and in some other experiments the lice appear to have been more readily infected after than during the first days of fever. It is interesting also that the box of lice fed on the day before the rise of temperature became infected.

Another interesting point comes out on examining this series, namely, that the lice in some of the boxes were more heavily infected than in others. This was shewn by differences in the proportion of lice found infected when films were made from single lice, and also by the fact that the excreta from two of the boxes shewed Rickettsia for only a few days, whereas most of the boxes which yielded infected excreta continued to do so as long as they were examined.

Thus, in box 268, fed on the day before the onset of fever, Rickettsia were found for two days only, and in another box, 271, only for six days. This latter box was fed on the second day of fever, and thus between two boxes, 270 and 272, neither of which ever yielded any Rickettsia. There is also a difference in the incubation in the louse before the Rickettsia appears: those boxes which are most infected appear to shew a shorter incubation as a rule.

A short period during which a box is infected is held to shew that only a small number of lice in that box were infected, and that these few died out early, leaving no further source for Rickettsia. The differences in the results of feeding on different days of illness are interpreted as indicating variation in the number of the Rickettsia in the peripheral circulation. Sufficient experiments have not been made to enable us to state definitely that the peripheral blood is always less infective for lice during the first two or three days of the disease, but such evidence as we have is in favour of this view. The two boxes fed daily became infected before the others, *i.e.*, both on the ninth day of the illness, and became the most heavily infected as shewn by the very large number of Rickettsia in the excreta and the high percentage (81 per cent.) of infected lice found in box 280. It is not necessary for our present purpose to recount further experiments in detail. It is enough to state that when lice are fed twice daily on a trench fever patient during the first week of his illness (and often much later), Rickettsia invariably appears in a proportion of the lice and their excreta after an interval of five to fourteen days, or rarely somewhat longer from the first infecting feed, if the lice are kept at a temperature of 27° to 32° C. between the feeds.

It has also been shewn that the excreta of lice fed in such a manner become infective for man after an interval of some days (about a week) from the first infecting feed.

For further details we must refer to the paper by Major Byam, R.A.M.C., and the Staff of the New End Hospital, Hampstead, read before this Society in May, 1918, and to a note by us in the *British Medical Journal*, of 21st September, 1918, and a more detailed account of our work in the *Journal of Hygiene* of April, 1919 (Arkwright, Bacot and Duncan, 1919).

### Association of Rickettsia with Typhus.

With regard to Rickettsia and typhus fever we have done comparatively little work, and for the evidence that this organism is constantly associated with the disease we are

mainly indebted to DA ROCHA-LIMA (1916), TÖPFER and others. We have, however, made a few observations, which are summarised below :—

1. Amongst a number of lice, *Pediculus corporis*, which had been fed on typhus fever patients, we found a louse containing large numbers of bodies closely resembling the Rickettsia bodies of trench fever.

2. Twenty lice from the same source were inoculated subcutaneously and intramuscularly into a *Macacus rhesus*, No. 14, which developed an illness with fever after twelve days' incubation, and died on the twenty-second day from the date of the infection ; no cause of death was found macroscopically post-mortem.

3. Another *M. rhesus*, No. 16, was inoculated subcutaneously and intramuscularly with 0·25 c.c. on 9th January, and with 3·5 c.c. on 10th January, of the blood of No. 14, taken on the seventh and eighth days of disease respectively.

No. 16 developed a well-marked febrile illness after five days' incubation.

4. A large number—about 150—monkey lice, belonging to the genus Pedicinus (probably *P. longiceps*. PIAGET) were taken from No. 14 after death, and were then emulsified in physiological saline solution and inoculated, on 13th January, subcutaneously and intramuscularly into No. 17. Also 20 to 30 pediculi fed on No. 14 from 9th to 11th January were fed on No. 17 from 13th to 21st January. This latter monkey, *M. rhesus*, had fever, beginning on 21st January, after seven days' incubation. It became very ill, and was killed on the tenth day of illness.

5. Two guinea-pigs inoculated with emulsions of spleen and supra-renal of No. 14 respectively shewed well-marked febrile reactions after nine and ten days.

6. Pediculi were fed on monkey No. 14 on the last (seventh, eighth, ninth) days of illness when the temperature was falling. The excreta from the box of lice examined on the sixth day from the first possibly infecting feed shewed Rickettsia bodies. The lice had been fed after the death of No. 14 on a fresh monkey, No. 17. Considerable difficulty was found in keeping pediculi alive which were fed on these monkeys

7. Pediculi were also fed on monkey No. 17 during the febrile period. Of four of these lice examined on the sixth to the ninth days from the first infecting feed, three were found to contain Rickettsia, or bodies which we take to be the Rickettsia, of typhus fever.

8. Lice (Pedicinus) native to the monkey, were taken from Nos. 14, 16, 17, and also from three normal control monkeys, and films were made from the alimentary canals of single lice and examined for Rickettsia. The results are shewn in Table II. :—

## TABLE II.

INFECTION OF MONKEY LICE (PEDICINUS SP.) WITH THE RICKETTSIA ASSOCIATED WITH TYPHUS.

| Monkey. | Day of Disease. | No. of Lice examined. | No. shewing Rickettsia. | Percentage. |
|---|---|---|---|---|
| No. 14 | 7th to 10th | 35 | 6 | 16 % |
| No. 16 | 9th to 17th | 26 | 1 | 4 % |
| No. 17 | 8th to 9th | 32 | 5 | 16 % |

*Control*—Lice taken from Normal Monkeys..

| Monkey. | No. of Lice examined. | No. shewing Rickettsia. |
|---|---|---|
| No. 10 | 38 | 0 |
| No. 29 | 20 | 0 |
| No. 31 | 17 | 0 |
| | 75 | 0 |

The Rickettsia which we have seen in lice, which have fed on typhus patients and monkeys, have closely resembled those seen in connection with trench fever, but so far as our observations go the following differences have been noticed :—

1. We have only once found the Rickettsia of typhus in the excreta of lice, though we have often looked for them.

2. The bodies found in connection with typhus stain a redder colour with Giemsa than those of trench fever, and sometimes appear slightly larger, and are more often lancet-shaped or like segmented bacilli in form.

### Sections of Lice.

Da Rocha-Lima (1916, 1917) and others have described Rickettsia bodies in very large numbers in the alimentary canal of lice fed on typhus and trench fever patients. It is stated that in microscopic sections these bodies can be seen within the epithelial cells of the gut in typhus but not, according to da Rocha-Lima, in trench fever.

In some of our sections of Pediculi from trench fever patients, and of Pediculi and Pedicini taken from typhus patients and monkeys, Rickettsia bodies are to be seen in enormous numbers lying close to the epithelium in the lumen of the midgut, and also in groups scattered through the altered blood which constitutes the gut contents.

In the material so far examined we have not seen definite invasion of the epithelial cells.

### Rickettsia in Rocky Mountain Spotted Fever.

Ricketts (1909) and more recently Wolbach (1916 and 1918) have described minute organisms in very large numbers in the alimentary canal and tissues of the tick. Wolbach also describes their presence in sections of the skin of human patients and in the skin and testis of infected guinea-pigs.

Through Dr. Wolbach's kindness we have been able to see films from the tick and sections of infected mammalian tissue. The organisms in the tick appear slightly larger than the Rickettsia of trench fever and typhus, and are usually longer and more lancet-shaped.

### Discussion.

The criticisms of da Rocha-Lima's work and of his interpretation of his observations have been brought along two main lines.

1. It has been maintained by some observers that these Rickettsia bodies are not micro-organisms, but granules of precipitated or coagulated protein, such as are found in old blood cultures.

2. It has been emphasised that these bodies were found by him and others in lice taken from persons who were free from any known contact with typhus.

3. A further criticism has been based on the earlier experiments of Nicolle, Conor and Conseil (1910), which seemed to shew that the virus of typhus would pass through a bacterial filter. The same argument against Rickettsia bodies being the virus of trench fever follows from the filtration work of the American Red Cross Committee on Trench Fever (1918).

The first criticism that the forms seen are not micro-organisms is upheld or discounted largely according to the personal opinion of the observer based on the microscopic appearance and the technique used in preparing the specimens. This criticism is most effective when the observer has himself made and stained his own films. However, if the view that the bodies are not a living virus is maintained it is necessary to account for their presence in infected lice and their excreta and not in normal lice nor in their excreta.

The second criticism that these bodies are found apart from typhus was to a great extent removed by the observation that similar forms occurred in lice which had fed on trench fever patients. Trench fever has been so frequent and widespread during the past few years in the war areas that it is not surprising that Rickettsia has been found in connection with persons other than typhus patients.

There still remain a few instances of lice from apparently healthy men in whom large numbers of Rickettsia bodies have been found by continental observers. Da Rocha-Lima (Munk and da Rocha-Lima, 1917), called the bodies in trench fever lice *R. quintana* and those in normal lice *R. pediculi*, to distinguish them from the bodies associated with

typhus, for which he retained the name *R. Prowazeki*. NICOLLE, BLANC and CONSEIL (1914) recorded finding the bodies described by SERGENT in 5 per cent. of the lice taken from persons in a district quite apart from any epidemic focus.

BRUMPT (1918) found Rickettsia in a high percentage of lice taken from seven prisoners of war at Rennes, who were apparently in good health, but he apparently only attempted to exclude contact with cases of typhus fever as he does not mention trench fever in his paper.

In London we have not so far found Rickettsia in lice from healthy civilians, and we attribute this to the practical absence of trench fever and typhus from the civilian population.

The claim that typhus fever virus is filterable was not confirmed by GOLDBERGER and ANDERSON (1912) and other workers. With regard to trench fever, four experiments on volunteers have been made by the War Office Committee with filtered emulsions of the infective excreta of trench fever lice. After filtering through a Chamberland F the filtrate failed to reproduce the disease in man.

It seems probable that the varying results under different circumstances are due to the size of the particles composing the virus. The Rickettsia bodies appear to be about 0·2 to 0·3 microns in diameter, *i.e.*, of such a size that during three hours' filtering some would occasionally pass through a filter which would keep back the *B. typhosus*, which was the test bacillus used to control the permeability of the filter by the American Committee.

Although this paper was written before its appearance, it is impossible to pass by without mention the paper recently published by ROSE BRADFORD, BASHFORD and WILSON (1919) in which it is claimed that the virus of trench and typhus fevers, among other diseases, has been cultivated in artificial media after passage through a Berkefeldt, and that these diseases have been reproduced by inoculation of the cultures. The work of these writers has not yet been published in sufficient detail to judge of its bearing on the rôle of Rickettsia in the diseases with which we are now concerned.

It seems that since the bodies which they describe are approximately of the same size as Rickettsia, the two forms may possibly be identical and if both are truly micro-organisms that their identity is probable

A difference in the two descriptions so far given is that while Rickettsia is by all observers stated to be readily decolourised by Gram's stain, the bodies of ROSE BRADFORD, BASHFORD and WILSON are Gram-positive. We have never seen Rickettsia retain the stain by Gram's method, although washing the film with ether has been tried as recommended by the last named workers.

It is perhaps unnecessary and premature at the present time to discuss the position of Rickettsia in a systematic classification. PROWAZEK and DA ROCHA-LIMA tend to the view that they are protozoa (Strongyloplasmata or Chlamydozoa). RICKETTS, WILDER, SERGENT, FOLEY and VIALATTE (1914) and TÖPFER appear to regard them simply as bacteria. WOLBACH apparently thinks that the micro-organism of Rocky Mountain fever which is found in the tick passes through slightly different forms in different phases of development. In any case it seems useful to retain the name Rickettsia for the present to denote the group of very small organisms including those of typhus and trench fevers, which, though to be seen in very small numbers in the blood of the infected mammalian host, are much more readily found in the invertebrate host which transmits the disease, and then are massed in enormous numbers in the alimentary canal and sometimes elsewhere.

## CONCLUSIONS.

*Trench Fever.*—The evidence for the association and causal connection of Rickettsia with trench fever may be summarised as follows:—

1. The organisms are constantly present in lice and the excreta of lice which have fed on trench fever patients, and are infective for man.

2. Their presence in these infective materials, usually in enormous numbers, corresponds to the minute dose, *e.g.*, the contents of the gut of one louse about 1 milligramme in weight, or a fraction of a milligramme of excreta required to infect a man.

3. Their appearance in the louse after an incubation period, usually of eight to ten days, which agrees roughly with the incubation period of the virus in the louse (five to twelve days). The virus was shewn to be present in these cases by human experiment.

4. The size of the Rickettsia is such that it would usually be held back by a bacterial filter, but may occasionally pass a filter which retains such bacteria as *B. typhosus*, or *B. prodigiosus*, and the virus also has been found to sometimes pass through a porcelain filter and sometimes not.

*Typhus Fever.*— The evidence of the connection of typhus fever with Rickettsia is of the same kind as in the case of trench fever, but the presence of the infecting virus can be shewn by the inoculation of monkeys and guinea-pigs. Our own observations lead us to the following conclusions:—

1. The Rickettsia of typhus does not appear in the excreta of lice with the same regularity as Rickettsia of trench fever.

2. Monkey-lice, *Pedicinus longiceps* (?) taken from a monkey infected with typhus on the seventh to the eighth day of the disease have been shewn to contain Rickettsia and probably typhus virus. Pedicini from monkeys which have not been inoculated have never, so far, been found to contain Rickettsia (75 lice examined).

3. Our comparatively few observations confirm the work of previous observers in the following particulars:—

(*a*) Pediculi fed on typhus patients may contain both the virus of typhus fever and Rickettsia.

(*b*) Both guinea-pigs and monkeys react with fever to the inoculation of typhus virus, whether in blood or lice or emulsions of organs taken post-mortem from animals.

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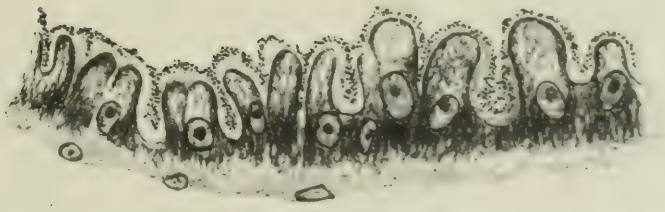
From excreta of lice (*Pediculus humanus*) fed on Trench Fever case.

× 1500.



From section through gut of louse (*P. humanus*) fed on Trench Fever case.

× 1000.

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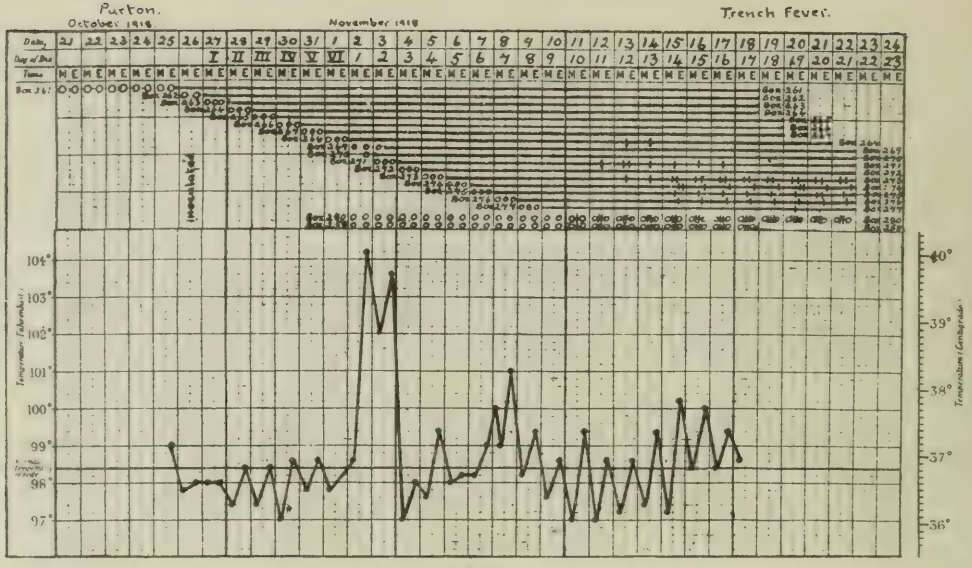



CHART I.

The lower part of the Chart shews the temperature of the volunteer Purton, who was inoculated with trench fever. Each of the horizontal lines (261 to 277, 280 and 258) in the upper part of the Chart represents a box of lice, and each sign O indicates that the lice in that box were fed on Purton ; the cross-marks, +, + +, on these lines shew the days on which the Rickettsia was found in the louse-excreta. The lines with no crosses represent boxes from which Rickettsia was never obtained. Boxes 280 and 258 were fed on Purton twice daily ; the boxes 262 to 277 were only fed on Purton on one day each ; box 261 was fed on Purton on the four days immediately before he was inoculated.

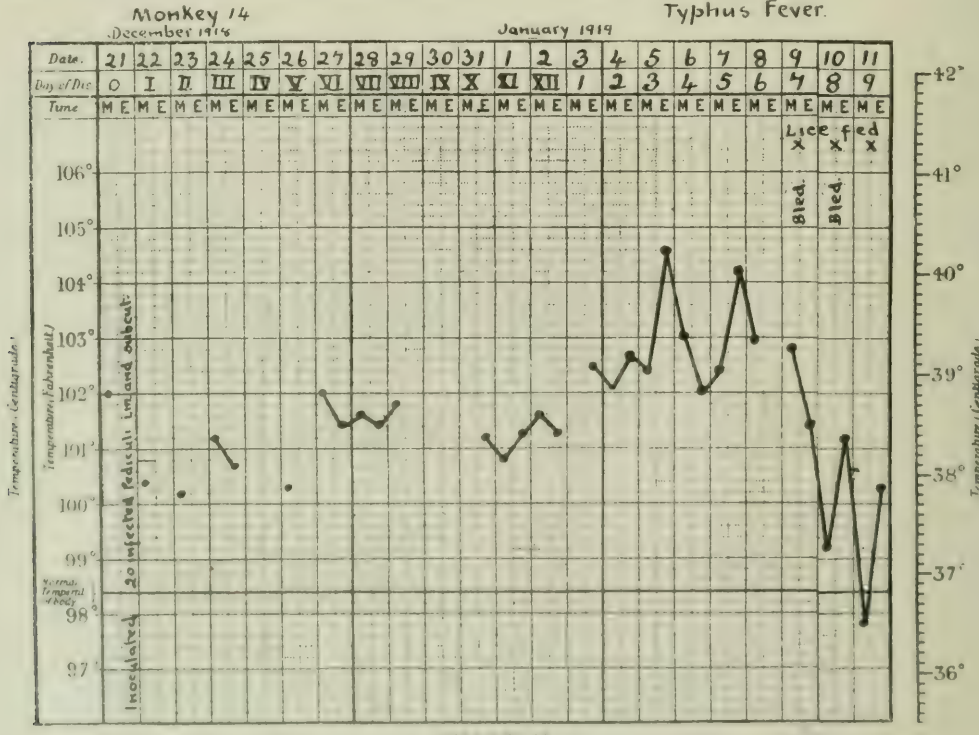



CHART II.

Monkey No. 14. *Macacus rhesus*, inoculated subcutaneously on 21st December, 1918, and intramuscularly with an emulsion of 20 *Pediculus corporis* from boxes which had fed on five human cases of typhus fever between the 5th and 15th Dec., 1918, on the 6th–11th and 4th–11th, 9th–15th and 11th–16th days of the fever respectively.

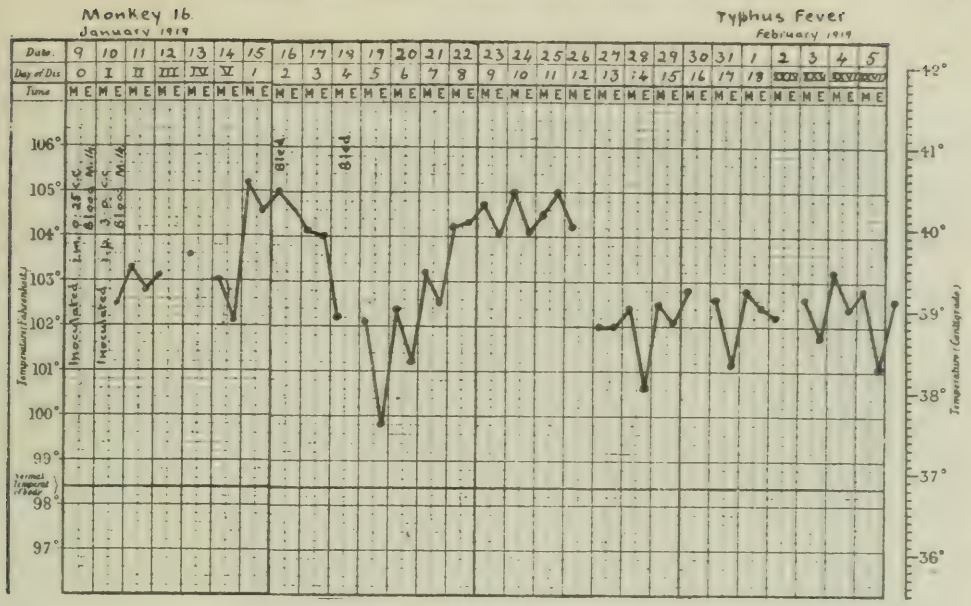



CHART III.

Monkey No. 16, *Macacus rhesus*, inoculated on the 9th January with 0·25 c.c. of blood, and on 10th January with 3·5 to 4 c.c. of blood from Monkey No. 14, taken on the 7th and 8th days from the onset of the disease in Monkey No. 14.

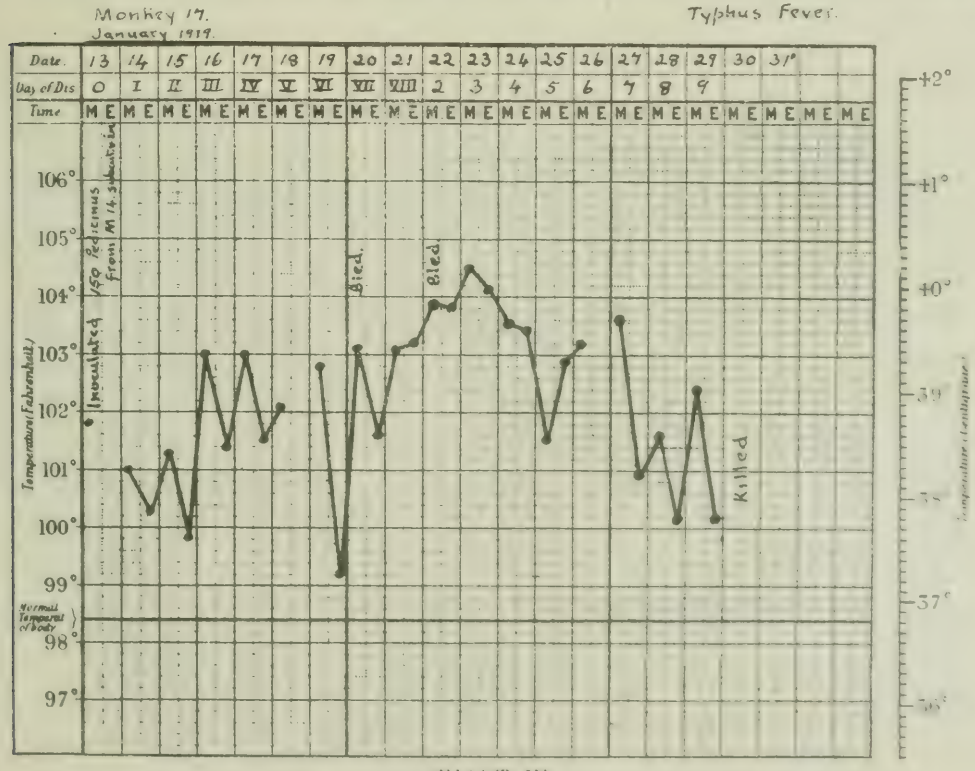



CHART IV.

Monkey No. 17, *Macacus rhesus*, inoculated subcutaneously and intraperitoneally with an emulsion of about 150 lice (*Pediculus*) taken from Monkey No. 14 on the 12th Jan. after death. Pediculi which had been fed on Monkey No. 14 on the 9th and 10th Jan. were also fed on Monkey No. 17 daily from the 15th to the 20th Jan.

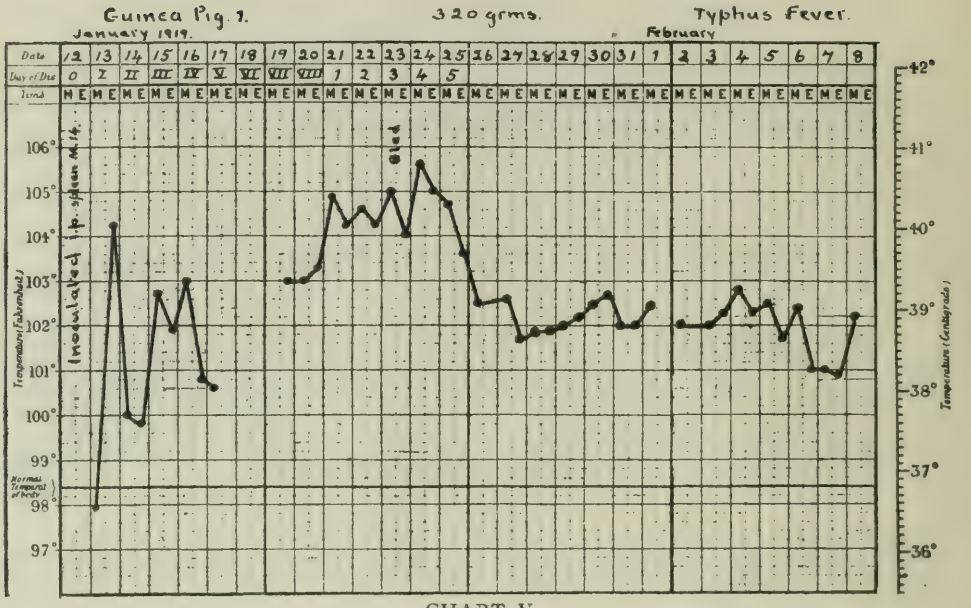



CHART V.

Guinea-pig No. 1, inoculated intraperitoneally with an emulsion of the spleen of Monkey No. 14.

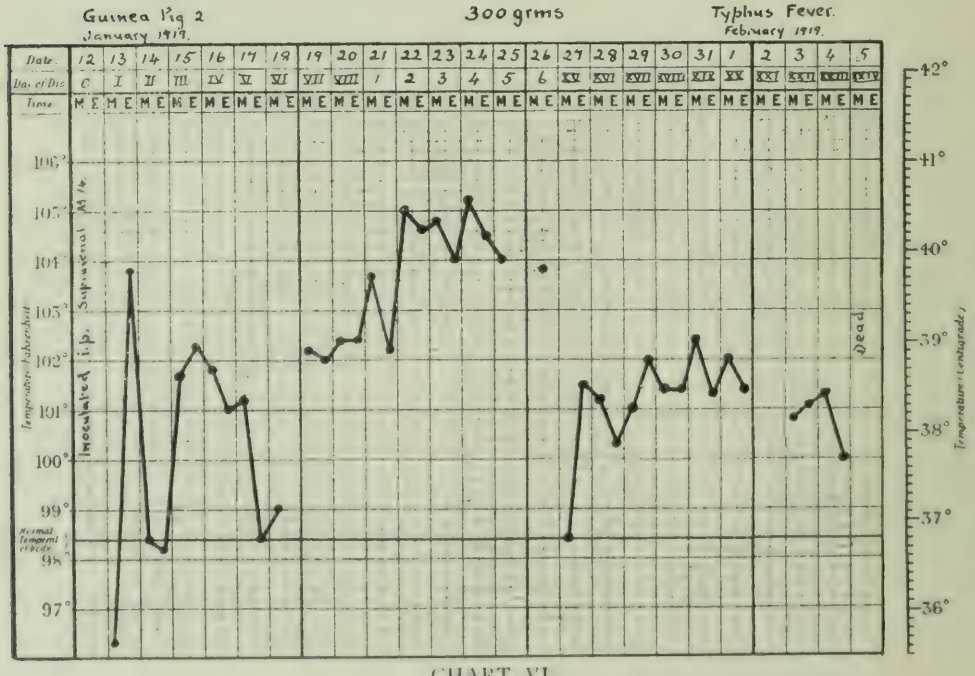



CHART VI.

Guinea-pig No. 2, inoculated intraperitoneally with an emulsion of the suprarenal of Monkey No. 14.

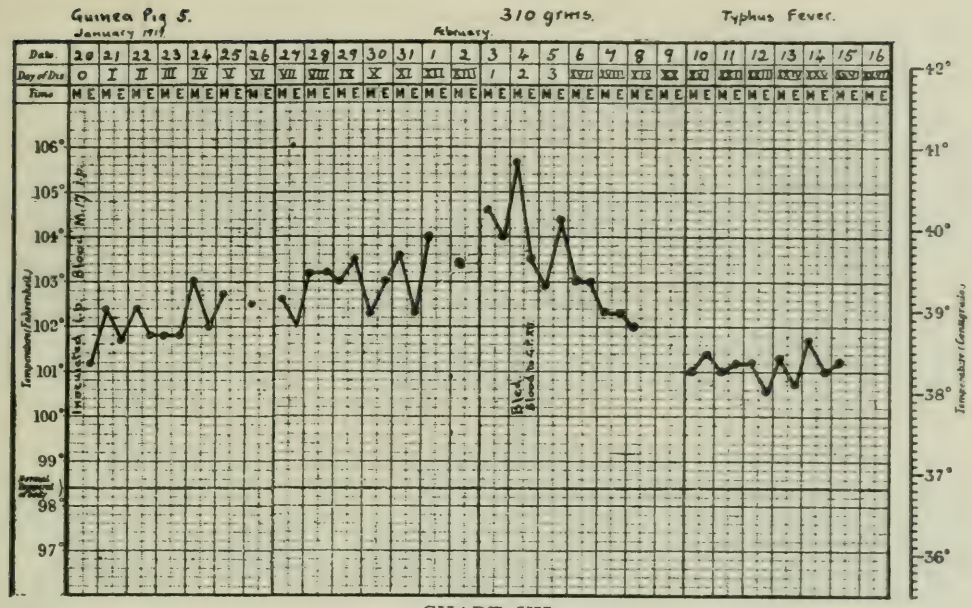



CHART VII.

Guinea-pig No. 5, inoculated intraperitoneally with 0·5 c.c. of citrated blood from Monkey No. 17.

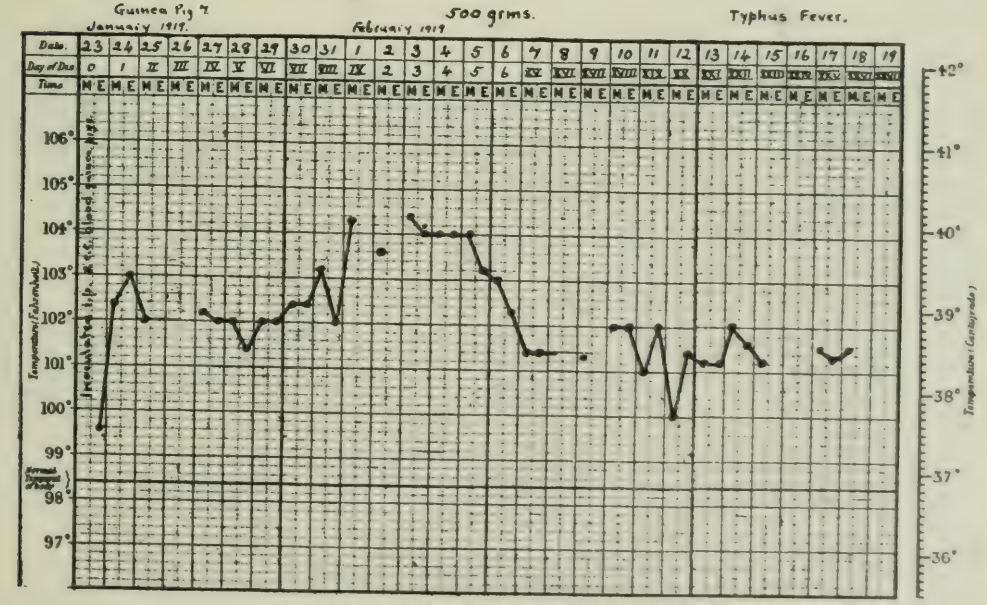



CHART VIII.

Guinea-pig No. 7, inoculated intraperitoneally with about 2 c.c. of citrated blood from Guinea-pig No. 1, taken on the third day of the fever.

# THE PATHOLOGY AND EPIZOOTIOLOGY OF LOUPING-ILL (DISEASE OF SHEEP). WITH SPECIAL REFERENCE TO CHROMATIN BODIES IN THE WHITE CORPUSCLES

By Sir STEWART STOCKMAN, *Chief Veterinary Officer Board of Agriculture and Fisheries.*

I have been engaged in the investigation of louping-ill for several years past. It is a seasonal disease and, therefore, one with regard to which investigation can only be pressed during a limited period of each year when it is in evidence. I will not attempt in a short space of time to go into every point which has been the subject of investigation, and I propose to limit myself to the results regarding what might be referred to as the fundamental points, making special reference to certain bodies, which I have provisionally called "chromatin bodies," present in the cells of lymphatic glands and of the blood of animals affected with the disease, as these may possibly occupy the important position of being the causal agent (full papers published in *Journal of Comp. Pathol. and Therap.* 1917 and 1918).

Some years ago I was able to shew by experimentation that British ticks could be infected by the piroplasm of the redwater of this country, and that this invertebrate host of the causal parasite could in a subsequent stage of its life cycle transmit the disease to other cattle, and further, that British ticks, given the opportunity, could become infected with, and be transmitters of, the tropical form of redwater, although the latter is caused by a different species of piroplasm. In pursuing these investigations, which involved innumerable examinations of blood from different animals, I also became aware that there exists an inoculable trypanosomiasis of British cattle, but the questions of whether it is insect-borne, and, if so, what insect is responsible, have not yet been solved. The point of these remarks in bringing to your notice another tick-borne disease of this country—a disease of sheep known popularly as "louping-ill" and "trembling" is to call attention to the following:—

As the investigation of diseases of animals proceeds it becomes more and more clear that diseases which are carried by invertebrate hosts, in which it is essential that the causal agents undergo part of their life cycle, are not by any means exclusively tropical or subtropical. It also appears probable that certain diseases classed as tropical could be established in this country.

Louping-ill causes very considerable losses on certain sheep farms, principally in the north of England and the south of Scotland, but it is by no means confined to these areas. It has been the subject of investigation by several pathologists working singly, and by a number of committees appointed by Government departments and agricultural societies.

Various claims have been made to the discovery of the cause of louping-ill, all of which have rested upon isolating a microbe from some part of the body of a sheep dead of the disease, and causing illness or even death with cultures of these microbes by inoculating them to other sheep. Such a method of investigation, particularly when applied to diseases of sheep, invites error, because in that species of animal cadaver-bacteria invade the tissues

with such extraordinary rapidity that they may be found in parts distant from the intestines in a few hours; indeed, transudates in the peritoneal cavity may even be invaded before death. None of the claimants to the discovery of these alleged causal microbes seem to have followed a methodical and obviously correct system of examining and inoculating the tissues or fluids of affected sheep obtained while alive, or from those which were slaughtered instead of being allowed to die, although recognising the nervous characteristics of the disease, portions of nerve tissues were frequently employed with negative results. Further, although the question of ticks being a factor in the causation of louping-ill has been discussed from time immemorial, both by farmers and by scientists, it seems always to have been dealt with in a somewhat partisan spirit by both, and was never submitted to investigation in the scientific sense, most preferring to accept it doubtfully, or reject it, entirely on the circumstantial evidence as it appealed to them individually. It is perhaps not surprising that the circumstantial evidence often gave rise to contradictory views, as the disease was originally identified by, and received its names from, farmers, the almost inevitable result being that different disease conditions were often grouped under the same name, and the epizootiological data were divergent.

Among the preliminaries which had to be undertaken in this investigation were:—

1. To select for the initial work only those cases in the field which might be looked upon as having a clinical or post-mortem entity; these were all collected from notoriously infected farms.

As regards macroscopic lesions the matter turned out to be simple in one sense, as it eventually became evident that the only really constant lesion was swelling and œdema of some of the lymphatic glands with sometimes congestion; this, however, in no way explained the symptoms. The possible bacteriological aspect of the disease was settled as regards microscopically visible microbes at a comparatively early stage of the investigation by finding that the fluids, tissues, and juices of animals slaughtered in various stages of the disease instead of allowing them to die were invariably sterile.

As regards clinical symptoms, it was found after careful sifting that cases could be grouped under three heads:—

(*a*) The animal may be found down in a senseless and completely comatosed condition with the head drawn round towards the shoulder, having lost all power to maintain itself on its legs, although the legs may be capable of performing paddling movements when the patient is laid on its side.

(*b*) The patient may be found down but quite sensible, the anterior, or posterior, or both extremities, being paralysed.

Along with these basic nervous symptoms others, such as general trembling, twitching of the muscles of the jaw, and frothing at the mouth, may be seen.

(*c*) It was said by many shepherds and flock-owners, and it agreed with my own observations, that every sheep reared on, or brought on to, louping-ill pastures passes through an attack of the disease in some form, and, if it survives, it becomes highly resistant to further attacks, at least in a dangerous form. Many sheep, however, never show objective symptoms visible to the ordinary observer, but shepherds and flock-masters of experience can pick them out as affected. This means that louping-ill may assume the form of a more or less slight indisposition.

(*d*) The indisposition may be somewhat greater than in (*c*) and the animal may lie down continuously apart from its companions, only rising at intervals to move a short distance, and then lie down again, or it may only move when chased.

For purposes of investigation, only cases shewing distinctive paralytic symptoms were chosen in the first instance, the object being to avoid as far as possible the inclusion of other diseases. When a lesion, such as an abscess, was found in a position in which it might possibly have caused paralytic symptoms, the case was also rejected. Following this method of eliminating more or less doubtful material it became apparent that a defined diseased condition existed, having for its distinctive symptoms those described under (*a*) and (*b*). On histological examination of the brains and spinal cords of such cases they shewed more or less definite lesions, which I need only refer to generally as those of

myelo-encephalitis. I may here add that the mild cases slaughtered for examination a a later stage of the inquiry shewed similar lesions in a lesser degree, and that the experimental cases to be referred to shewed the same lesions.

2. It seemed important to sift, analyse, and make further observations in connection with the various statements regarding the circumstantial evidence for and against the tick as a factor. This resulted in the adoption of the following definite conclusions :—

(*a*) Ticks are always present on the pastures where true louping-ill is present, although it has been asserted that this is not so. It is true, however, that the disease may be unknown on land infested by ticks, but this may only mean that the ticks on such pastures have never had the chance of becoming infected.

(*b*) That louping-ill is a seasonal disease occurring chiefly in April, May and early June and to a less extent in autumn, and that these times are times when ticks in various stages, but ready to feed, are most plentiful.

Let us return for a moment now to the most constant macroscopic lesion, namely, the œdematous lymph glands. On cutting into these a very thick creamy juice was found in the medullary portion. Smears made from this creamy material when stained by Giemsa stain shewed the chromatin bodies to which I will later refer more particularly. The condition of the glands and the presence of these bodies suggested that inoculation with this material might be followed by interesting results. It may be said here, however, that material from the œdematous glands, the blood, and other tissue juices was all examined carefully by dark-ground illumination for motile bodies with negative results; the only motile bodies found being gyrating particles indistinguishable from those seen in normal blood and tissue juices.

An affected lamb (21) was sent up alive to the laboratory from an infected farm which was being used as a field observation station, On arrival it shewed the disease in one of its distinctive forms, its hind legs being completely paralysed. It was killed by bleeding. All the organs and tissue juices proved sterile on media. Microscopical lesions of the cord (examined at a later date) were evident; the cells of the œdematous glands and the blood shewed chromatin bodies. (The lesions of the cord were illustrated on screen.)

*Experiments 52-55*—Two ewes and two lambs were inoculated subcutaneously with 5 per cent. of an emulsion from the œdematous glands of the above lamb. One ewe (12a) became distinctly ill on the eleventh day and died paralysed on the thirteenth. The other ewe (14) on the twelfth day had become dull, listless and disinclined to move, but it had apparently recovered on the sixteenth day. One lamb (11a) became ill on the ninth day and died paralysed on the twelfth. The other lamb (13) became ill on the ninth day and died paralysed on the twelfth. The three animals which died shewed typical lesions in the cord and chromatin bodies were present in the swollen glands. The bodies were present in blood smears from all four inoculated animals, one of which did not die.

(These are only selected experiments for illustration, others were shewn on the screen.)

These results were not accepted as conclusive at this stage because of certain apparent failures at the time to carry on the disease by inoculation in series.

## Tick Experiments.

The sheep tick which prevails exclusively on infected farms is the *Ixodes ricinus*. I have made many observations on the habits of this tick as seen on louping-ill pastures, but with only a few of these need I take up your attention. The *I. ricinus* is known as a thrice sucking tick, which leaves its host and moults on the ground each time after engorgement, that is to say, it requires three vertebrate hosts for its full life-cycle. The prevalence of this or that stage on the pastures, then, at a particular time of the year depends to some extent on whether its hosts are available to enable it to pass from one stage to another. On these sheep pastures larvæ, nymphæ and adults are exceedingly plentiful in April and May. All forms are again found in August, September and October, but not so plentifully. These, as already mentioned, are the louping-ill seasons, but the main damage is done in the spring, because of the advent of new lambs which provide fresh and suscep-

tible fuel for the disease, most of the old stock having become highly resistant by the autumn. If sheep of any age from clean farms be introduced at these seasons, however, the losses may be enormous. Experiments were carried out with ticks which in some previous stage had fed upon an infected sheep in the field. The first results were apparently negative in the sense that no distinct objective symptoms arose, but as temperatures were not at first taken, it may be that reactions actually occurred. On account of the strong circumstantial evidence, the inoculation results, and the sterility of the tissues and fluids, however, the tick experiments were persisted with from season to reason.

*Experiment 73 (Larvæ)—Lamb 299 (Sample experiment).*—Between two and three thousand larvæ—*I. ricinus*—the progeny of females which had engorged on affected sheep in the field in May, were put on the scrotum of this lamb at the laboratory in September. On the sixth day the lamb was shewing uneasiness, constantly getting half up on its knees, and then lying down again. No particular attention was paid at first as the symptoms were not distinctive, and no temperatures were taken. On the seventh day the lamb was unexpectedly found dead. The lesions were typical of louping-ill.

*Experiment 74—Lambs 292a and 289 (Larvæ and sub-inoculation with blood).*—On the 19th September, 1916, two or three thousand larvæ of the same origin as those used on Lamb 299, were put on the scrotum of Lamb 292a.

On the fourth day the lamb was panting and uneasy, and seemed distinctly ill, lying for long periods, and only moving when made to get up, but showing nothing like complete paralysis. Smears from the blood shewed chromatin bodies; cultures from the blood were sterile.

Lamb 289 was inoculated into the muscles with 10 c.c. of blood drawn from lamb 292a when the temperature of the latter was 107°. A reaction occurred to 107°. This lamb was killed by bleeding for examination, and lesions referable to myelo-encephalitis were found on histological examination.

*Experiment 82—Lambs 447 and 454.*—On August 10th, one adult male and two female ticks were put to feed on lamb 447. The ticks had engorged as nymphs on a sheep affected with acute louping-ill in the field in May of the same year. They had moulted at the laboratory towards the end of July. Only one tick was observed to engorge on 447. The temperature chart (on screen) shews the reaction up to 107° which occurred. The usual lethargic symptoms appeared and were very evident on the twelfth day, but there was no definite paralysis. Chromatin bodies were found in the mononuclear cells in blood smears taken during the reaction. It appears from this experiment that the disease can also be carried by adult ticks which as nymphæ engorged on affected sheep, and incidentally it may be noted that in the case of adult ticks at least gross infestation is not a condition necessary to infection. Lamb 454 received subcutaneously 80 c.c. of blood taken from 447 when its temperature was 107.4°. The chart (on screen) shews a reaction up to 107.6°. The blood shewed chromatin bodies.

Having obtained these results with ticks and with blood of sheep infected by ticks it was indicated to inquire more methodically whether blood drawn in life from sheep while suffering from an attack of the disease was infective; a necessary factor to ticks becoming carriers is that an infecting agent is available in the blood of the vertebrate host.

*Experiment 87 (Selected)—Ewe 317 and others.*—This experiment, which included a considerable number of animals, is shewn in graph form on the screen.

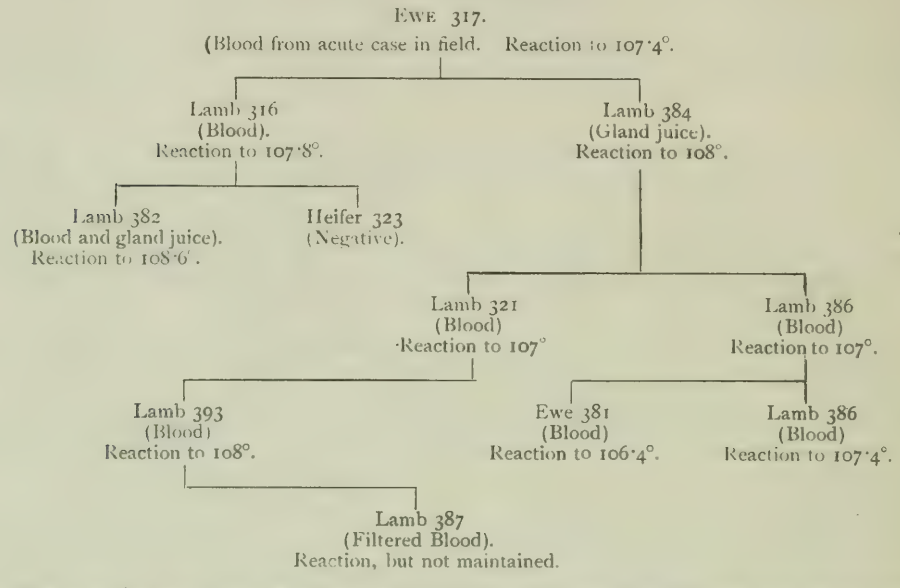
Ewe 317 was inoculated with about 200 c.c. of blood, obtained during life, from an acute and ultimately fatal case of louping-ill in the field. The graph shews that the disease can be transmitted in series from susceptible animal to susceptible animal, provided the proper materials—blood and gland juice—are used for inoculation, meaning that a living virus and not a toxin is involved. It also shews a negative reaction on the part of a heifer.

With regard to lamb 387—filtration experiment—it received 1,000 c.c. of blood which had been diluted for filtration, corresponding to about 50 c.c. of undiluted blood.

The rise of temperature to 106·4° occurred on the following day, and was almost

certainly due to the large amount of material which was injected into the hind leg, which caused lameness. After the second day it fell to normal.

GRAPH OF EXPERIMENT 87.




The negative result as regards material after filtration was obtained on several occasions with blood. More definite information, however, seemed desirable on the question of whether the virus was filterable or not. Accordingly a further experiment was designed, using an emulsion of œdematous lymph glands in which the virus seems to be specially concentrated.

*Experiment 95—Ewe 461 and lamb 448.*—Immediately after slaughter several glands, all more or less œdematous, were removed from a wether which was passing through a reaction after being inoculated with virulent blood. One gland from the lumbar region was slit open with a sterile knife, and a few drops of the thick juice were removed and transferred to a tube of sterile broth. The remainder of this gland and two others were cut up into small pieces, bruised in a mortar with a small amount of salt solution, and the resulting emulsion was passed through a Berkefeldt filter.

Ewe 461 received subcutaneously 5 c.c. of the unfiltered broth emulsion (reaction to 107·2°). Lamb 448 received subcutaneously 25 c.c. of a similar emulsion after filtration. (No reaction.) Charts shewn on screen.

Lastly, it remained to inquire whether ticks could be infected on the experimental sheep which were reacting to inoculation with blood or gland juice.

*Experiment 96 Lamb 452.* In the month of May, larvæ and nymphæ were put to engorge on a sheep while reacting to blood inoculation. The blood causing the reaction was drawn from another sheep which was reacting to blood obtained from a case of natural infection in the field. The ticks engorged, and moulted to nymphs and adults, as the case might be, by August. Some of these (nymphs and adults) were put to feed on Lamb 452 (Charts shewn on screen, reaction to 108°). Chromatin bodies were found in the mononuclear cells of the blood on the seventh day after tick infection, the temperature then being 108°.

Many other questions in connection with this interesting disease have been inquired

into; for example, it appears that after reacting to blood or lymph gland inoculation sheep are highly resistant to further inoculation. Sheep inoculated in the laboratory have also been exposed on infected pastures, and have come through a louping-ill season.

Again the question of whether ticks after infecting an animal become harmless has also been inquired into. The evidence so far obtained seems to shew that once having bitten on the sheep an infected tick loses its infectivity, but further work is being carried out on these questions.

Practical methods of prevention and eradication have also been studied, and it seems probable that the disease can be dealt with by either methods of prevention or eradication, which are practicable.

The result of the investigation might be summarised as follows:—

1. The blood and the juices of the œdematous glands from sheep affected with louping-ill in the field cause symptoms and lesions identical with those of louping-ill, the microscopic lesions being describable as a myelo-encephalitis as in natural cases; the blood and gland juices of the experimentally inoculated sheep are similarly virulent.

2. The larvæ from female ticks of infected sheep in the field, and adults which as nymphæ engorged on such sheep can convey the disease to other sheep: this means that infection passes through the egg. Similarly, ticks fed on sheep when reacting to inoculation with blood or gland juice may be infective to other sheep.

3. As the virus can be transmitted in series by inoculation, it follows that it is a living agent, and from the fact that it is transmitted by the tick it is almost certainly a protozoon parasite.

4. The causal agent is apparently not ultra-microscopic.

* * * *

It is the question of the causal agent to which I wish to draw your particular attention. If smears be made from the juice of the œdematous glands and stained by Giemsa's method, a particularly large number of what appear to be disintegrating nuclei cells are sometimes present. (Preparations were shewn under the microscope and on the screen). A number of the large and a few of the small mononuclear cells contain grouped or scattered bodies which stain a deep crimson-lake colour throughout. They vary from small dots under the oil immersion lens to objects of considerable size. They do not appear to have a highly differential structure, but in shape they may be round, oval, pyriform, or bacillary. They are mainly confined to the cell protoplasm, but in a few cases similar forms are to be seen in the nucleus against the lighter lilac tint of that body. The number of separate bodies in a single cell varies from one up to half a dozen or even more. Sometimes they are collected in nests giving the appearance of division having taken place, and fine strands of chromatin may be seen connecting two particles. In some of the cells the protoplasm may shew one or two clear or bluish spaces limited by a definite margin giving the appearance of a cyst, and inside are to be found a varying number of chromatin bodies of different sizes and shapes. By focussing carefully, a dark spot can sometimes be made out inside the bodies, but they stain so deeply that it is difficult to say whether a nucleus is present. The cell nucleus may be wholly or partially destroyed, the individual bodies being set free to enter or be taken up by other leucocytes, but for the purposes of interpretation it is important to note that many of the cells containing chromatin bodies are quite intact as regards the nucleus and cell protoplasm. In blood smears made from a sheep suffering from acute louping-ill, or during a definite febrile reaction following upon inoculation with blood from a case of louping-ill, large mononuclear leucocytes containing similar oval and bacillary bodies are often particularly evident at the margin of the smears. As far as it is allowable to attribute a development to these bodies, or attempt to trace it by examination of fixed smears, it might be described as follows:—

A single oval body penetrates the leucocyte in which it increases in size and becomes more open like a rosette. It then appears to be become fragmented into smaller parts like the original, and divides up continuously. With a certain amount of reason one

might construct a parasite of the hæmogregarine type (leucocyte variety) out of these findings, some of the bodies in the glands being regarded as trophozoites and merozoites, and the individual particles in the mononuclears of the blood stream as trophozoites and gametocytes. On the other hand, the chromatin particles, particularly those in the cells of the blood stream, might be nothing more than the debris of chromatin material which has been phagocytosed. It might also be suggested with a certain amount of reason that the chromatin particles result from chromatolysis of the nucleus, but in this connection it should be remembered that some of the cells containing the bodies are normal in every respect except for their presence. It is not claimed, however, that a final statement can be made on the evidence so far obtained that these chromatin bodies are parasitic in character, and the causal agent of louping-ill. Further investigation into their character is being carried out, but for purposes of discussion it seems allowable to summarise the evidence in favour of the parasitic view :—

(1) As has been shewn, the disease is tick-borne.

(2) The fluids and organs of sheep containing the bodies, and only such material, produce the disease by inoculation.

(3) The bodies stain somewhat after the manner of parasites.

(4) The causal agent of the disease is apparently not ultra-microscopic, since it is retained by filters as coarse as the Berkefeldt, and the chromatin bodies referred to are the only abnormal objects observable under the microscope in infective material.

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## Discussion.

Surg.-Capt. P. W. Bassett-Smith : The work done by Dr. Arkwright has been most thorough, and the results are most interesting ; but, as it is so late, there is not time to discuss the various controversial points. The way that he has met the continental criticisms, especially those of Brumpt, are valuable, the infected pediculi used by the latter being derived from German prisoners, which, however, did not pass on trench fever, as might have been expected.

With relation to trench fever, Dr. A. C. Coles has sent a communication of work done, and has shewn me spirochætal forms in films of blood taken from successfully experimentally inoculated cases which were sent to him by Major Byam, R.A.M.C., from the Hampstead Hospital, by permission of Sir David Bruce.

The spirochætal bodies were found after prolonged search in the blood films of two out of six cases, in the first paroxysm of the fever. Photomicrographs were exhibited.

The spirochætes all stain a delicate blue with Giemsa, and are faintly granular, and vary considerably in form. Dr. Coles states that they cannot be regarded merely as artefacts, but the fact that they were detected only in definite cases of artificially induced trench fever, and then only during the first attack, is at least suggestive. These findings, in conjunction with the work done at the Pasteur Institute, should encourage still further research as to the spirochætal origin of trench fever.

Professor W. J. Simpson : It is very difficult to collect one's thoughts and do justice to the papers that have been read without having had an opportunity of seeing and studying them beforehand. Indeed it would be an advantage if such a plan could be adopted. The two papers represent much the same stage of research in their respective spheres. Sir Stewart Stockman shews the presence of chromatin bodies in great numbers in louping-ill, and establishes the fact that the infection is continued from one generation to another, and that these infected ticks produce the disease. The veterinary department have the advantage over the medical department in being able to conduct their experiments to a final conclusion. Sir Stewart Stockman hopes to be able to isolate these chromatin bodies from the juices and any unseen organisms that may possibly be present in these juices, and we shall look forward to his further contribution on the subject.

Dr. ARKWRIGHT's description of the discovery of the Rickettsia bodies in trench fever and in the louse, and the rôle that the louse plays in trench fever, demonstrate that this investigation has reached a very advanced stage, and it is to be hoped that the next will be the isolation and cultivation of these bodies, and the production of the disease with the cultures. There are many pitfalls, however, in such investigations, and one has to be particularly cautious. I have fallen into the pitfalls myself. I have been interested in the cultivation of vaccine nearly all my professional life, and when in India succeeded in cultivating from a buffalo with rinderpest, but which, in this instance, I consider, was a form of smallpox, a minute diplobacillus, which produced in calves a vaccine vesicle, the lymph of which produced vaccine vesicles in other calves and children, who were thereby rendered immune against subsequent vaccination with ordinary vaccine lymph. I lost the strain of culture in the rains. I have never been satisfied in my mind whether the minute diplobacillus was the actual causal agent, or whether it was merely the visible companion of a more minute and invisible organism in symbiotic relation with it. Such a possibility has always to be borne in mind in these investigations. I would ask the Fellows to accord a hearty vote of thanks to the authors of the papers read this evening.

# HAS EMETINE ANY INFLUENCE ON THE SCHISTOSOMES?

BY

C. BONNE, *Paramaribo, Dutch Guiana.*

In March, 1916, I treated an East Indian immigrant, who suffered from amœbic dysentery, with emetine. The patient received each day a subcutaneous injection of one grain of emetine hydrochloride on five successive days, with the usual good effect on the dysenteric symptoms. The stools of this man had been containing also ova of *Schistosomum mansoni*, and I was rather astonished when I could not find these eight days afterwards. I repeated the examination on sixteen following days always with the same negative result.

This made me try a series of five injections of one grain of emetine with other patients shewing the eggs in the fæces, the results of which, together with those of the first patient, are given in the table below. Each time at least three slides and, as a rule, 4–12 were examined either by myself or by trained assistants when the eggs could not be found. In several of the cases I put a premium on the finding of the eggs, which, as the table shews, I often lost in the end.

The results can be summarised as follows:—

1. As a rule the eggs disappear from the stools after about a week. In a few cases they do not disappear, but they are found less frequently (12, 16, 18), or even this is not obvious (19); but then they may still disappear after a second course of injections (16, 18).

2. After about two months, sometimes sooner, already the eggs are found again, but can be made to disappear by a second course of injections.

Point 1 can be explained as follows: It takes about a week for the eggs to work themselves out of the tissues into the lumen of the rectum, and no new eggs are laid in the wall of the rectum by the worms for some reason, *e.g.*, they may be dead or have moved away from the veins there to the larger veins of the portal system.

Point 2 can be explained by the worms returning to the rectum wall, either the old ones or other worms, *e.g.*, younger worms, which had not yet left the portal vein when the injections were given. The reappearance of the eggs is too common to be explained by reinfection.

Schistosomiasis is not a severe disease in Dutch Guiana, at least not in the very large majority of the cases. Nearly all the patients of the Table shewed no intestinal symptoms at all, and the eggs were only found in the routine examination of the fæces. When they had intestinal symptoms, dysentery like symptoms, for instance, I very often found amœbæ or dysentery bacilli by which they could be explained. I believe that the presence of the schistosomes, probably the passage of the eggs through the mucosa, predisposes to infections with dysenteric organisms. On the other hand, I met with cases where no such organisms could be found and still there was passage of mucus and blood and abdominal pain. The emetine has a beneficial and often a very striking effect on these symptoms. This is readily understood when amœbæ are present, but in the other cases there must be some other action on the diseased mucosa. It is always possible, however, that a small number of amœbæ were at work but not discovered. I

think one must not too hastily ascribe all sorts of abdominal troubles to the schistosomes, when eggs of *Schistosomum mansoni* are found. There is no doubt, of course, that in some cases the worms are able to cause serious damage. In Dutch Guiana, however, this is rare. I never met with a case of death due to schistosomiasis, although in the course of three years more than two hundred cases have entered the hospital in Paramaribo, nearly all of them for other diseases. Patient number 4 of the Table had a definite polyposis recti, and a few of the tumours removed by operation contained a great number of the eggs.

It was necessary of course to control the patients during a long time, and it was very difficult to get the fæces of these people for examination when they had left the hospital. This was one of the reasons why I did not continue these controls. Then I did not like the explanation of the disappearance and reappearance of the eggs, because it was so hypothetical, and I saw no chance to prove or to disprove it. It was desirable to know whether the emetine killed the worms in the rectum wall or not, and this could only be proved by a post-mortem shortly after a course of injections. I never had such a chance. It would be worth while to know, if one could be freed permanently of the eggs in the fæces by an intermittent treatment with emetine. But as the persons that I saw harbouring the worms had not suffered from them for a long time, they declined to receive these repeated injections. It is even possible that there was no influence on the schistosomes at all, and merely on the mucosa through which the eggs have to pass. The results obtained, however, can hardly be ascribed to mere chance.

Diamantis had some results with emetine in bilharzial hæmaturia in Egypt (*Trop. Dis. Bull.*, Vol XI. No. 2). Martin Meyer noticed improvement in a patient suffering from urinary and intestinal bilharziasis, and Van Hoof in a patient with rectal bilharziasis (*Trop. Dis. Bull.*, Vol. XII., No. 3). It was only after I had read the results of these authors that I thought it worth while to publish my own experiences. They leave no doubt that emetine has an influence on the symptoms in rectal schistosomiasis due to *Schistosomum mansoni*, but I cannot say whether the drug is able to kill off the worms.

On the following page is a Table shewing the influence of emetine on the presence of eggs of *Schistosomum mansoni* in the stools :—

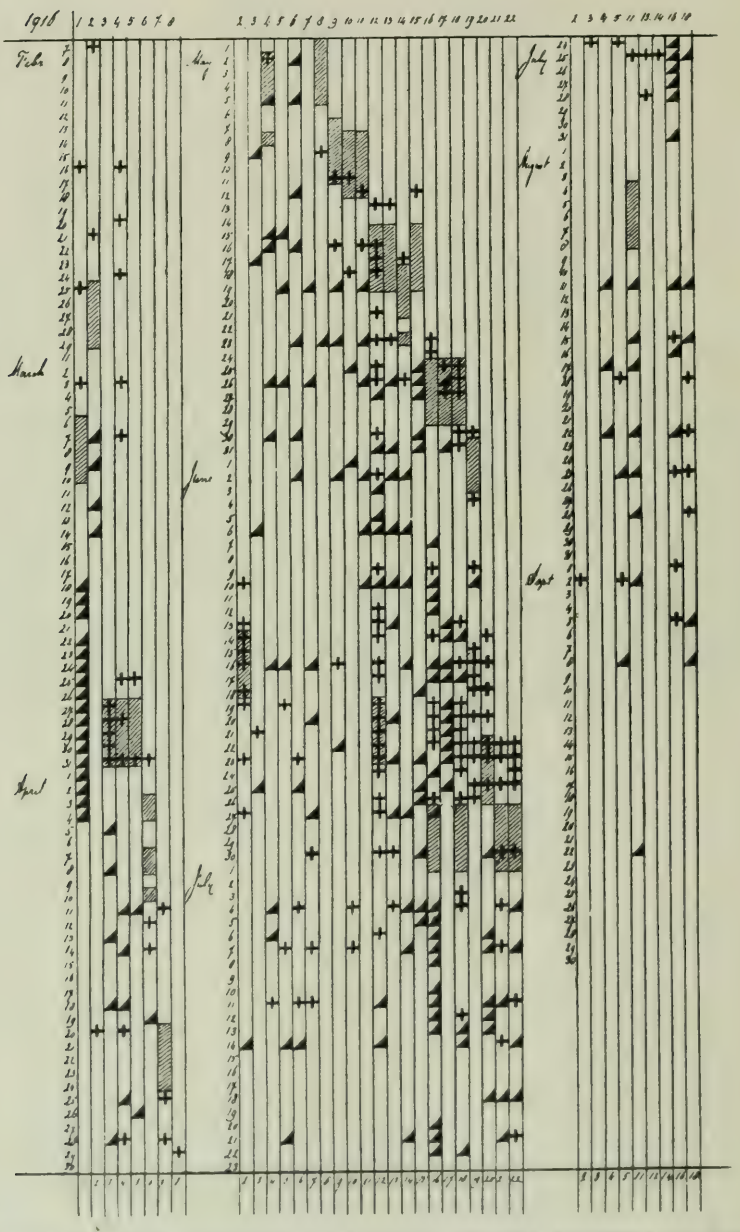

TABLE SHEWING THE INFLUENCE OF EMETINE ON THE PRESENCE OF EGGS OF SCHISTOSOMUM MANSONI IN THE STOOLS.

+ means eggs found. ◢ means eggs not found. ≡ period of emetine injections.

RAPHAËL BLANCHARD.

# Obituary.

## RAPHAËL ANATOLE EMILE BLANCHARD

(1857–1919),

HONORARY FELLOW OF THE SOCIETY.

RAPHAËL BLANCHARD was born February 28th, 1857, at Sainte-Christophe (Indre-et-Loire), in France.

His father, RENÉ BLANCHARD, who died at the early age of twenty-six, was a poet and author. The driving force which distinguished RAPHAËL from the start is, perhaps, best exemplified by reference to his grand-uncle, JEAN PIERRE BLANCHARD (1753–1809), the aeronaut, who first devised the parachute for use in aeronautics in 1785. JEAN tested the apparatus on a dog enclosed in a basket, and, in 1793, himself descended from a balloon by this means, although he broke his leg in the proceeding. He was the first aeronaut to make a voyage across the British Channel, flying from Dover to France in a balloon (7th January, 1785).

In 1874 RAPHAËL BLANCHARD went to Paris to take up the study of medicine and natural science, and in 1875 he became assistant to CHARLES ROBIN. In 1876, with others, he founded the Societé Zoologique de France, of which he became the Secretary-General and moving spirit for twenty years. The year 1877–78 was spent by him in Austria and Germany, after which he returned to Paris and became assistant to PAUL BERT (1878–83). In 1878 he taught natural history at the Lycées Saint-Louis and Louis-le-Grand, and in 1884 gave instruction at the Ecole d'Anthropologie. In 1883 he became Associate Professor at the Paris Faculty of Medicine, and started the first courses of Parasitology that were delivered in France. In 1889, with MILNE EDWARDS, he organised the International Congresses of Zoology, in which he took an active part, especially in establishing the Rules of Zoological Nomenclature that have since been so widely adopted. It was owing to his energetic efforts in this direction that he was made President of the International Commission on Zoological Nomenclature in 1898. In 1894 he became member of the Academy of Medicine, and in 1897 was elected full Professor of Medical Zoology, the title of this chair being changed nine years later to that of Parasitology at his own request. With others, in 1902, he founded the Societé Française d'Histoire Médicale, of which he was president during the first three years. In the same year was inaugurated the Institut de Médecine Coloniale, with its hospital, whose foundation was brought about by BLANCHARD, with the aid of funds supplied by the Association des Dames Françaises. He was Annual Secretary of the Academy of Medicine from 1912 until his death. Throughout the war he gave devoted service to his country in promoting more particularly the work of the Association des Dames Françaises.

BLANCHARD'S published work ranged over a wide field. A full account of it will be found in a series of brochures, entitled *Notices sur les Titres et Travaux Scientifiques de M. le Dr. Raphaël Blanchard*, the first of which was published at Lille in 1890, whilst two supplements appeared in Paris in 1893 and 1908; a third and final supplement is now being prepared for press. The complete lists of publications contain no less than 603 titles of books, brochures, original papers, short notes, articles that appeared in encyclopedias, circulars, etc., some of which were translated into various languages from the French. A good list of his publications, covering nine pages, will also be found in Stiles and Hassall's *Index Catalogue of Medical and Veterinary Zoology* (Washington, 1902).

His publications relating to Parasitology are the most important; they deal with parasitic worms, leeches, protozoa, moulds, myiasis, injurious or disease transmitting

insects, mosquitoes, acari, pseudo-parasites, myriapods, and touch on various tropical diseases. He published numerous papers on zoological nomenclature, general zoology: on comparative anatomy, histology, physiology, pathology and teratology. He likewise ventured into the domain of anthropology, wrote on medical history, education, and on such miscellaneous subjects as numismatics (he possessed an extensive collection), book plates, and sundials!

Of the larger works may be mentioned the *Elements de Zoologie* (written with PAUL BERT, 1885), his *Traité de Zoologie Médicale*, in two volumes (1885–1890); the *Histoire naturelle et médicale des moustiques* (1905), the *Epigraphie médicale*, of which four fasciculi have appeared since 1907, this work dealing with epitaphs, etc., having medical interest. Finally must be mentioned the *Archives de Parasitologie*, a quarterly journal founded by him in 1898, and that he edited and owned.

The foregoing summary indicates sufficiently the remarkable character of BLANCHARD's activities. He was an indefatigable research worker, a good organiser, a highly gifted and versatile man. All who knew him felt that he was the embodiment of energy and enterprise. He had a considerable command of languages, and was an eloquent speaker, qualities which rendered him eminently fitted to attend international congresses. He participated frequently at these meetings, and his well-known shining figure will be greatly missed at future congresses of medicine and of zoology. He had many warm friends; that he should have had some enemies is natural, for such is the fate of most leaders of men.

RAPHAËL BLANCHARD died on February 7th, 1919, at the age of 62 years, with a suddenness that came as a great shock to all his friends. He had had what he supposed to be merely a severe cold, and which gave those about him little concern. He was arranging to begin his lectures on February 10th, when suddenly, without warning, he had an attack of syncope and passed away.

It seems fitting to conclude this obituary notice with a passage translated by the writer from a short biography written by LINOSSIER (*Paris Médical*, 15 fév., 1919) a few days after his friend BLANCHARD's death:—

"BLANCHARD led a brilliant existence. Life smiled upon him. He knew little of its bitterness, and he had a lively appreciation of its joys. Honours were showered upon him, and he received them willingly. He had arrived at the summit of his ambitions. Then death, seeing him happy, and fearing for him the inseparable afflictions of the finest old age—sadness and melancholy—desired to shield him from them: considerately and gently she spared him the agony of her approach—suddenly she struck him down by a touch of her wing, and bore him away tenderly without giving him pain. . . ."

*20th April, 1919.* G. H. F. NUTTALL.